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DNA-dependent SUMO modification of PARP-1

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ABSTRACT

Poly(ADP-ribose) polymerase 1 (PARP-1) plays an important role in DNA repair, but also contributes to other aspects of nucleic acid metabolism, such as transcriptional regulation. Modification of PARP-1 with the small ubiquitin-related modifier (SUMO) affects its function as a transcriptional co-activator of hypoxia-responsive genes and promotes induction of the heat shock-induced *HSP70.1* promoter. We now report that PARP-1 sumoylation is strongly influenced by DNA. Consistent with a function in transcription, we show that sumoylation *in vitro* is enhanced by binding to intact, but not to damaged DNA, in a manner clearly distinct from the mechanism by which DNA damage stimulates PARP-1's catalytic activity. An enhanced affinity of PARP-1 for the SUMO-conjugating enzyme Ubc9 upon binding to DNA is likely responsible for this effect. Sumoylation does not interfere with the catalytic or DNA-binding properties of PARP-1, and structural analysis reveals no significant impact of SUMO on the conformation of PARP-1's DNA-binding domain. *In vivo*, sumoylated PARP-1 is associated with chromatin, but the modification is not responsive to DNA damage and is not affected by PARP-1 catalytic activity. Our results suggest that PARP-1's alternative modes of DNA recognition serve as a means to differentiate between distinct aspects of the enzyme's function.

1. Introduction

The biological properties of a protein can be altered by post-translational modification with small chemical groups, such as phosphate, or entire polypeptides, such as ubiquitin and the small ubiquitin-related modifier, SUMO [1]. In this way, post-translational modification systems impinge on their targets without the need for protein synthesis, and are consequently able to mediate rapid responses to changes in the cellular environment. Like other members of the ubiquitin family, SUMO controls a variety of cellular pathways, ranging from nuclear import to transcriptional regulation [2,3]. A prominent role in DNA metabolism is emerging from an involvement in several DNA repair systems [4]. In many cases, SUMO modulates the interactions of its targets with other cellular proteins by creating a binding site for a SUMO-binding motif within the interaction partner [2,5]. However, SUMO conjugation can also alter the DNA-binding properties of its targets. This is observed with the base excision repair factor Thymine DNA Glycosylase (TDG), where sumoylation causes the release from an abasic site within a stretch of double-stranded (ds)DNA [6–8], and with the recombination factor Rad52, where sumoylation reduces the affinity for both single-stranded (ss) and dsDNA [9]. Conversely, DNA binding can induce the modification of a target protein. For example, loading onto DNA strongly stimulates the sumoylation of the budding yeast replication clamp, PCNA, *in vivo* and *in vitro* [10], and similarly, ssDNA enhances the sumoylation of Rad52 in a purified system [9]. Like ubiquitylation, SUMO conjugation is mediated by a cascade of activating enzyme (E1), conjugating enzyme (E2) and ligase (E3), with Ubc9 acting as the sole E2 and playing a major role in substrate recognition by means of a direct binding motif, ΨKX(D/E) (where Ψ stands for a bulky aliphatic residue: reviewed by [11]). Although many SUMO E3s harbour DNA-binding domains that might explain a preferential sumoylation of DNA- or chromatin-associated targets, the above-mentioned cases likely involve changes in the properties or conformations of the target proteins themselves, as the DNA-mediated stimulation of their sumoylation does not require the presence of an E3 [9,10].

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Another post-translational modification with an impact on DNA metabolism is mediated by the family of poly(ADP-ribose) polymerases (PARPs) [12]. PARPs catalyse the transfer of ADP-ribose moieties from NAD^+ onto target proteins to form linear or branched chains. PARP-1 is the most abundant member of the family and at the same time the major acceptor of poly(ADP-ribose) in the cell [13,14]. It is best known for its role as a damage sensor, as its binding to damaged DNA strongly stimulates its catalytic activity and thereby promotes the cellular response to DNA single- and double-strand breaks (SSBs and DSBs) [15]. Recognition of unusual DNA structures, such as nicks and breaks but also loops and junctions, is mediated mainly by an N-terminal DNA-binding domain (DBD) that contains two zinc finger motifs, F1 and F2 (Fig. 1A). Although F1 does not bind DNA very strongly, it is essential for activation of PARP-1 by SSBs and DSBs [13,15–17]. In contrast, F2 has a high affinity for DNA containing nicks or breaks, but is dispensable for DSB recognition [16,18–21]. A third (structurally unrelated) zinc-coordinating domain, F3, is also involved in the stimulation of PARP-1's catalytic activity upon binding to damaged DNA [22–24]. A central automodification domain (AD), encompassing a BRCT domain for protein–protein interaction, is followed by the C-terminal region containing a WGR domain that collaborates with F1 and F3 in DNA binding and makes extensive interdomain contacts [20], and the catalytic fold (CAT).

In addition to its functions as a DNA damage sensor, PARP-1 plays a role in regulating gene transcription both under basal conditions and in response to specific signals such as hormones, cytokines, Ca^{2+} and heat shock [25]. Its contributions to transcriptional regulation are mediated in several different ways: although PARP-1 can act as a promoter-specific co-regulator and by binding to specific enhancer sequences and insulators, its predominant function in transcription can probably be attributed to a modulatory influence on chromatin structure. In fact, poly(ADP-ribose) is recognised by specific chromatin-remodelling factors, but it can also directly affect chromatin compaction state [26–30]. Interestingly, not all of PARP-1's effects on transcription require its catalytic activity [25,31].

PARP-1 has been identified as a SUMO target [32–35]. Modification of human PARP-1 by the SUMO isoforms SUMO-2 and SUMO-3 occurs predominantly at two sites, K203 and K486. These modifications do not affect PARP-1's catalytic activity, but appear to impinge on its transcription-related functions [32,33]. Messner et al. [32] demonstrated that PARP-1 sumoylation reduces the expression of hypoxia-responsive genes in low-oxygen conditions, likely due to an inhibition of the interaction between PARP-1 and the acetyl transferase CBP/p300. In contrast, Martin et al. [33] showed that PARP-1 sumoylation contributes to heat shock-induced activation of the *HSP70.1* promoter. They also found that PARP-1 sumoylation was up-regulated following heat shock, leading to its poly-ubiquitylation by the SUMO-targeted ubiquitin ligase RNF4 and subsequent proteasomal degradation. Finally, Ryu et al. [34] reported an accumulation of SUMO-2/3-modified PARP-1 on mitotic chromosomes in *Xenopus laevis* egg extracts and suggested a function for this modification event in controlling PARP-1's activity towards other chromatin-associated proteins.

In a search for chromatin-bound SUMO conjugates in *X. laevis* egg extracts, we independently identified PARP-1 as a target of SUMO-2 (our unpublished data). In order to gain insight into the nature of a putative cross-talk between the DNA-dependent poly(ADP-ribosyl)ation and sumoylation systems, we have investigated how DNA structure affects PARP-1 sumoylation. We demonstrate here that sumoylation is strongly stimulated by intact DNA, but is not induced by DNA lesions. Our results lend support to a DNA damage-independent function of PARP-1 sumoylation and suggest a mechanism for how the distinct functionalities of the polymerase could be delineated through its interactions with relevant forms of DNA.

2. Materials and methods

2.1. Proteins and antibodies

Recombinant PARP-1 F1 + F2 and PARP-1 F2 (WT and R122I) were produced and purified as described [18]. Full-length PARP-1 (WT, K203R, K486R, K203/486R and C298A) was produced as an N-terminal His_6-fusion in insect cells from pDEST10 derivative constructs and then purified by chromatography using 3-aminobenzamide-sepharose 4B affinity resin [36], HiTrap Heparin and Superdex 200 (GE Healthcare). N-terminally His_6-3C-tagged SUMO-1 (WT and T95R) was produced in bacteria from pET15 derivative vectors and isolated by Ni-NTA affinity resin (Qiagen) and gel filtration (Superdex 200). Untagged SUMO-1 was generated in bacteria from pET11 derivative plasmids [37] and purified by ultrafiltration (MWCO 30,000, Vivaspin) and gel filtration (Superdex 75). His_6-tagged SUMO E1 was purified according to Yunus and Lima [38]. Untagged SUMO E1 was purchased from Boston Biochem. Untagged Ubc9 was purified as described [37]. ^{GST}Ubc9 was produced in *Escherichia coli* and purified on glutathione Sepharose (GE Healthcare). Recombinant human ^{GST}RanGAP1 was from Novus Biologicals.

PARP-1 and the F1 + F2 fragment were detected by western blot using mouse monoclonal antibody F1-23 (Enzo Life Sciences). SUMO-1 and SUMO-2/3 were detected by polyclonal, affinity-purified antisera raised against His_6-tagged recombinant proteins. Antibodies against FLAG- and His_6-tags were from Sigma, those against GST, GAPDH, poly(ADP-ribose) and histone H3 from Molecular Probes, AMS Biotechnology, Trevigen and Millipore, respectively.

2.2. Cell culture

All cell lines were maintained in DMEM supplemented with 4.5 mg mL^{-1} glucose, 2 mM glutamine, 15 μg mL^{-1} phenol red (11995-040, Invitrogen), 10% (v/v) heat-inactivated foetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. For detection of sumoylation sites in PARP-1, 60% confluent HEK293 cells in 35 mm ⌀ dishes were co-transfected with 1 μg each of pSG5-His_6-SUMO-1 [39] and pDEST-PARP-1-$FLAG_3$ (WT, K203R, K486R, K233R, K249R, K512R, K203/486R, K233/486R, K249/486R, K486/512R, K305/486R, K352/486R, or K442/486R) with 6 μL of Fugene HD (Roche). Cells were analysed 48 h post-transfection. For detection of endogenous sumoylated PARP-1, HeLa cells carrying a stably integrated tetracycline-inducible His_6-SUMO-3 construct [40] were grown in 150 mm ⌀ dishes to 40% confluency and incubated in the presence or absence of 2 μg mL^{-1} doxycycline for 24 h before being exposed to the relevant conditions. For experiments involving PARP-1 inhibition, cells were incubated with or without 10 μM inhibitor (PJ34, Sigma) for 1 h before treatment with H_2O_2 (10 mM, 5 min) or camptothecin (1 μM, 2 h). For cycloheximide chase analysis, a tetracycline-inducible PARP-1 construct (WT or K203/486R) was stably integrated into HEK293 cells. After seeding ~5×10^5 cells into 60 mm ⌀ plates and growth for 24 h, cells were induced with 1 mg mL^{-1} doxycycline for another 24 h before washing and treatment with 50 μg mL^{-1} cycloheximide in the absence or presence of 30 μM MG132.

2.3. In vitro sumoylation assays

Sumoylation reactions were generally performed in 20 mM HEPES-KOH pH 7.5, 110 mM KCH_3CO_2, 2 mM $Mg(CH_3COO)_2$, 0.05%

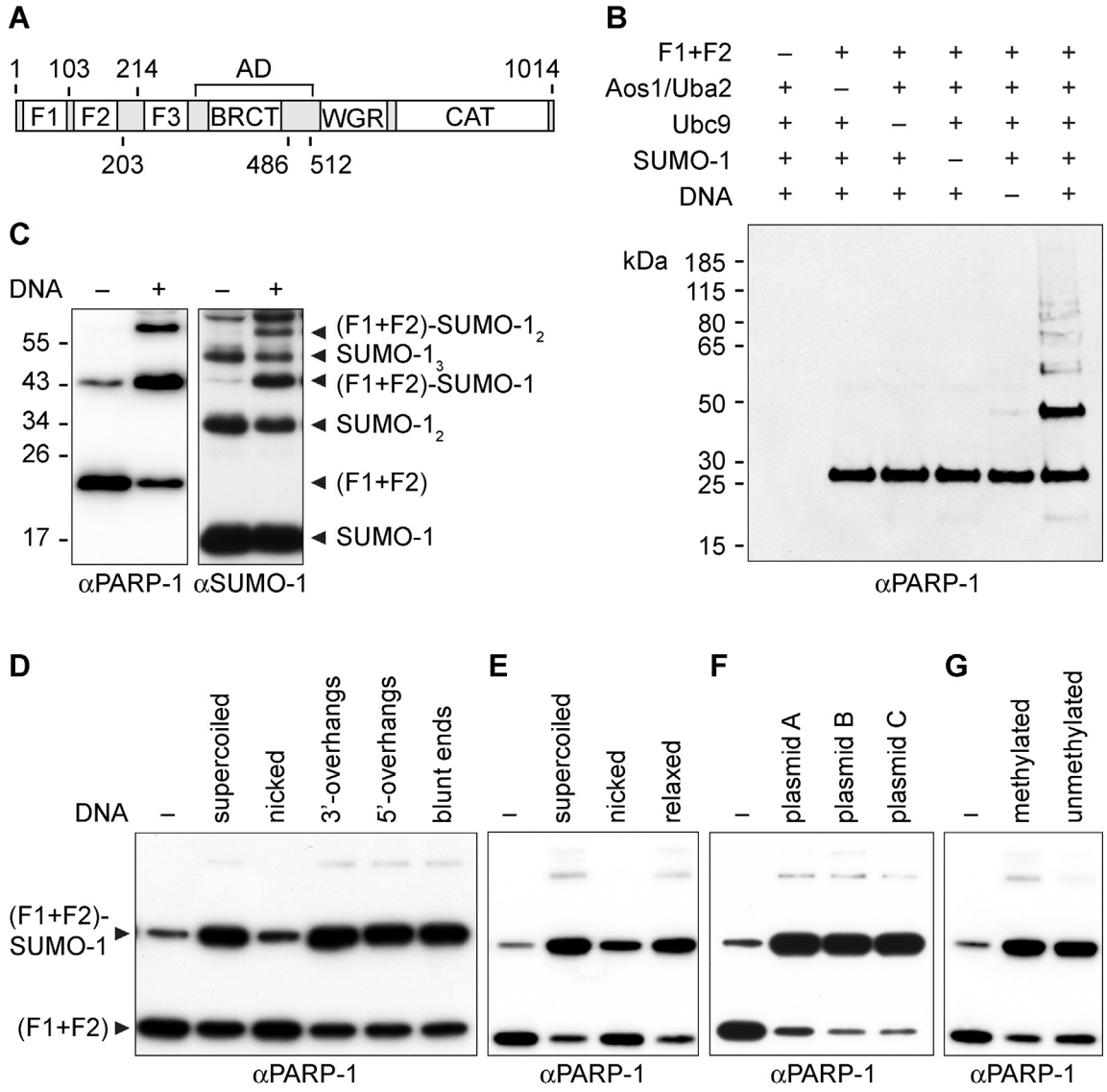

Fig. 1. Sumoylation of PARP-1 F1 + F2 is stimulated by DNA. (A) Domain structure of PARP-1, indicating relevant domains and amino acids. F1–F3: Zn-coordinating domains; AD: automodification domain; CAT: catalytic domain. (B) *In vitro* sumoylation of F1 + F2 is strongly stimulated by DNA. Reactions were performed under standard conditions in the presence or absence of 150 ng supercoiled plasmid DNA. (C) Addition of heavily digested plasmid DNA (240 ng ≈ 360 pmol bp) stimulates sumoylation of F1 + F2 (3 pmol), but not the formation of free SUMO-1 chains. Western blots developed with anti-PARP-1 and anti-SUMO-1 antibodies show the respective conjugates. (D) Influence of SSBs and DSBs on the efficiency of F1 + F2 sumoylation. Enzymes: nicked – Nb.BsrDI (5 sites); 3′-overhangs – PstI (2 sites); 5′-overhangs – EcoRI (2 sites); blunt – EcoRV (2 sites). (E) Influence of supercoiled versus relaxed DNA on the efficiency of F1 + F2 sumoylation. Relaxation was achieved by topoisomerase I treatment. (F) Influence of different DNA sequences on the efficiency of F1 + F2 sumoylation. (G) Influence of DNA methylation on the efficiency of F1 + F2 sumoylation. The same plasmid was isolated from *E. coli* K12 or a *dam*$^-$ *dcm*$^-$ derivative, respectively.

(v/v) Tween 20, 0.2 μg μL^{-1} BSA and 1 mM DTT buffer containing: 25 nM SUMO E1 (His_6-tagged unless otherwise stated), 150 nM Ubc9, 300 nM SUMO (His_6-tagged unless otherwise stated), 150 nM of the relevant substrate and 2 mM ATP. The type and amount of DNA included in the reactions are indicated in the relevant figure legend. Analytical reactions were carried out in a total volume of 20 μL, 3 μL of which were loaded onto either a 10% (w/v) (PARP-1 F1 + F2) or 6% (w/v) (full-length PARP-1) polyacrylamide gel. For mass spectrometry, the reaction was scaled up to 45 mL for PARP-1 F1 + F2 (6.75 nmol together with 330 μg supercoiled plasmid DNA), and 1.3 mL for the full-length protein (200 pmol His_6-PARP-1, untagged E1 and SUMO-1^{T95R} and 15.6 μg supercoiled plasmid DNA). For NMR analysis, the reaction was scaled up to 1.4 L (200 nmol ^{15}N- and ^{13}C-labelled PARP-1 F1 + F2 and 10 mg supercoiled plasmid).

2.4. In vitro interaction assays

DNA-binding assays were performed with 60 pmol of 15 bp duplex DNA (biotinylated on one strand). Unlabelled DNA served as a negative control. The DNA was incubated with 20 pmol of protein (^{GST}Ubc9 or F1 + F2) in 100 μL of binding buffer (20 mM Tris–HCl, pH 8.0, 125 mM NaCl, 1 mM $MgCl_2$, 0.1% Triton, 1 mM DTT) on ice. The mixture was then added to 10 μL of streptavidin agarose that had been blocked with 5 mg mL^{-1} BSA in binding buffer before, and incubated with rotation at 4 °C for 1 h. The beads were washed three times with 150 μL of binding buffer, and bound material was eluted by boiling in SDS loading buffer. Binding of F1 + F2 to SUMO was examined in the same buffer as above, but containing 50 mM NaCl. Proteins (20 pmol F1 + F2 or ^{GST}Ubc9 as control) were incubated with 10 μL of SUMO-2 agarose (Boston Biochem) in the presence

or absence of 500 ng plasmid DNA with rotation at 4 °C for 1 h before washing three times with buffer and eluting bound material by boiling in SDS loading buffer as above. Interactions between Ubc9 and F1 + F2 were assayed in the same way, using 60 pmol ^{GST}Ubc9 or GST and 20 pmol F1 + F2 or ^{His}SUMO-1. Protein mixtures were incubated on ice for 1 h and added to blocked glutathione Sepharose beads, which were then processed as before. Bound material was analysed by western blotting with relevant antibodies.

2.5. Purification of sumoylated proteins

Sumoylated PARP-1 (F1 + F2 and full-length) was purified from reactions performed in the absence of BSA or Tween 20. Following the addition of NaCl and imidazole to a final concentration of 500 mM and 20 mM, respectively, the reaction was passed over a HisTrap HP column (GE Healthcare). For sumoylated PARP-1 F1 + F2, the resin was washed in 50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 20 mM imidazole, 1 mM DTT, exchanged to the same buffer but containing 120 mM NaCl, incubated with GST-tagged rhinovirus 3C protease at a protease:SUMO molar ratio of 1:40 overnight at 4 °C, eluted through a GSTrap HP column to remove the protease, and resolved by gel filtration on a Superdex 75 column equilibrated in 50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT. For sumoylated full-length PARP-1, the HisTrap HP resin was washed with 20 mM HEPES-KOH, pH 7.5, 500 mM NaCl, 20 mM imidazole, 1 mM DTT, eluted with the same solution but containing 400 mM imidazole, dialysed into 100 mM NH_4CH_3COO and concentrated to 50 μL by ultra-filtration (MWCO 30,000).

2.6. Preparation of DNA ligands for sumoylation reactions

Sequences of the oligonucleotides used to assemble the various DNA ligands are given in Table S1. The dumbbell structure has been described previously [18]. Plasmids were derivatives of pCR4-blunt-TOPO (A, 4.8 kbp, Invitrogen), pET3 (B, 5.3 kbp, Merck) or pDEST32 (C, 5.5 kbp, Invitrogen). Histone octamers were assembled on a 11 kbp long DNA molecule consisting of a tandemly repeated 187 bp long high-affinity histone octamer binding sequence [41].

Digested and nicked plasmids (11 μg) were prepared by incubation with 110 U of the relevant enzyme (New England Biolabs) for 3 h at 37 °C (or 65 °C for nicking) in 120 μL of the appropriate buffer. Relaxation of a supercoiled plasmid (5 μg) was brought about by 5 U of topoisomerase I (Invitrogen) for 3 h at 37 °C in 500 μL of 50 mM Tris–HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 30 mg ml^{-1} BSA and 0.5 mM DTT. These DNA molecules were cleaned up with Qiagen's QIAquick PCR Purification Kit. Oligonucleotides (50 μM in 50 mM Tris–HCl, pH 7.5, 250 mM NaCl) were annealed by incubation at 98 °C or boiling water for 3 min followed by slow cooling to room temperature. The nicked dumbbell (20 μM) was sealed with 2000 U of T4 DNA ligase (New England Biolabs) for 16 h at 25 °C in total volume of 40 μL. The reaction was terminated by heating to 65 °C for 5 min.

2.7. Mass spectrometry

Sumoylated PARP-1 (F1 + F2 and full-length) was subjected to denaturation, reduction, alkylation and digestion by trypsin (Promega) in the Janus Liquid Handling System (PerkinElmer). Peptides were analysed on a SYNAPT HDMS mass spectrometer (Waters). The data were analysed against the UniProt database by means of the MASCOT algorithm (Matrix Science) using a precursor tolerance of 5 ppm, a fragment ion mass tolerance of 0.8 Da, one missed trypsin cleavage site and variable modifications. MS/MS data were manually curated and validated using the Scaffold programme (Proteome Software).

2.8. NMR spectroscopy

^{15}N- and ^{13}C-labelled sumoylated PARP-1 F1 + F2, sumoylated with unlabelled SUMO-1, was analysed in 50 mM Tris–HCl, pH 7.0, 200 mM NaCl, 150 μM $ZnSO_4$, 4 mM [$^2H_6$] DTT and 5% (v/v) D_2O using a DMX600 spectrometer (Bruker) equipped with a triple resonance (^{1}H/^{15}N/^{13}C) cryoprobe. Data were acquired at 300 K and ^{1}H, ^{15}N and ^{13}C chemical shifts (δ) were calibrated using sodium 3,3,3-trimethylsilylpropionate (Sigma) as an external ^{1}H reference. Spectra were analysed with either TOPSPIN (Bruker) or SPARKY (Goddard, T.D. and Kneller, D.G., University of California, San Francisco). Signals were assigned by analogy with the previously assigned F1 + F2 fragment [18]. Amide group chemical shift perturbations between the unmodified PARP-1 F1 + F2 and sumoylated PARP-1 F1 + F2 were calculated using the formula $\Delta\delta = \sqrt{((\delta^1H)^2 + (\delta^{15}N/10)^2)}$.

2.9. Detection of sumoylated PARP-1 in cultured cells

Unless otherwise noted, cells producing His_6-SUMO ($\sim 10^7$) were resuspended in 1 mL of pulldown buffer (6 M guanidine hydrochloride, 100 mM sodium phosphate, pH 8.0, 300 mM NaCl, and 10 mM Tris–HCl, pH 8.0, 15 mM imidazole, 0.15% Tween 20) containing 2 mM β-mercaptoethanol, incubated at room temperature for 1 h with rotation, sonicated for 20 s at 20% power on a Branson Sonifier and supplemented with 20 μL of Ni-NTA resin. After an overnight incubation at room temperature, the resin was washed three times in pulldown buffer containing 20 mM imidazole, and twice more in 8 M urea, 100 mM sodium phosphate, pH 8.0, 10 mM Tris–HCl and 30 mM imidazole, before bound material was analysed by gel electrophoresis and western blot. For cell fractionation, cells were lysed by resuspension in 10 mM HEPES, pH 7.9, 0.1% Triton X-100, 10 mM KCl, 1.5 mM $MgCl_2$, 340 mM sucrose, 10% glycerol, 1 mM PMSF, 5 μg/mL aprotinin and 20 μg/mL leupeptin, and incubation on ice for 10 min. Samples were centrifuged at 1300 × *g* for 5 min, and the supernatant was used as the soluble fraction. The pellet was resuspended in 20 mM Tris–HCl, pH 8.1, 2 mM EDTA, 500 mM NaCl, 0.1% SDS, 1% Triton X-100 with Complete™ protease inhibitor and incubated on ice for 10 min, followed by sonication. After centrifugation at 16,100 × *g* for 5 min, the supernatant was used as the chromatin fraction. For Ni-NTA pulldown experiments, each fraction was combined with pulldown buffer at a ratio of 1:1. Whole cell input samples were prepared in NET-N lysis buffer (1% NP-40) by sonication.

2.10. PARP-1 activity assays

Catalytic activity of PARP-1 was determined using a colorimetric assay kit with immobilised histones as substrate (Trevigen). Assays were performed according to the manufacturer's instructions, but exchanging the supplied control DNA for double-stranded oligonucleotides of varying lengths and amounts as indicated.

3. Results

3.1. Sumoylation of the PARP-1 DNA-binding domain is affected by DNA

Detection of SUMO-modified PARP-1 predominantly in the chromatin fraction ([33,34] and our unpublished results) prompted us to examine the effect of DNA on PARP-1 sumoylation *in vitro*. For these assays, we used SUMO-1 in order to minimise the extent of poly-SUMO chain formation. In order to further reduce the complexity of the experimental system, we used an N-terminal fragment of PARP-1 that is liberated upon caspase cleavage *in vivo*

(aa 1–214) as a substrate for *in vitro* modification assays. This fragment, named F1 + F2, contains the two zinc finger motifs that have been implicated in DNA binding and damage sensing and one of the physiological sumoylation sites, K203 (Fig. 1A). *In vitro* sumoylation of this protein by the E2 Ubc9 was strongly enhanced by the addition of DNA (Fig. 1B and C). In contrast, the formation of free SUMO chains or modification of an unrelated substrate, RanGAP1, under the same conditions remained unaffected by DNA (Figs. 1C and S1), indicating that the stimulatory effect of DNA was specific for F1 + F2. Mass spectrometry confirmed that K203 served as the SUMO acceptor site *in vitro* (Fig. S2).

Given that structural features of DNA such as nicks and breaks strongly activate PARP-1's catalytic activity, we asked what types of DNA structures would most effectively stimulate F1 + F2 sumoylation. To our surprise, we observed that a circular supercoiled plasmid enhanced the modification efficiency to the same degree as digested plasmid preparations containing blunt ends, 3′- or 5′-overhangs (Figs. 1D and S3). A plasmid treated with a nicking enzyme to introduce single-stranded breaks (SSBs) did not stimulate the reaction. In contrast, relaxation of supercoiled plasmid DNA with topoisomerase I did not abolish its capacity to stimulate F1 + F2 sumoylation (Figs. 1E and S3). This result indicates that the observed difference between supercoiled and nicked DNA was due to the presence of SSBs rather than the absence of torsional strain or potential secondary structures such as loops or cruciforms that might result as a consequence of supercoiling. Stimulation was comparable with three different plasmids of minimal sequence identity but similar size (Fig. 1F), indicating that the observed effect was unlikely due to specific sequences. Finally, the presence or absence of the N^6-adenine and C^6-cytosine methylation patterns found in *E. coli*-derived DNA did not affect the ability of the DNA to stimulate the reaction (Fig. 1G). Taken together, these results indicate that F1 + F2 sumoylation can be promoted in a sequence-independent manner by various forms of DNA, including a relaxed circular plasmid without apparent secondary structural features. However, those structures that most effectively stimulate PARP-1's catalytic activity, i.e. SSBs, not only fail to activate sumoylation, but apparently even inhibit the stimulatory effect of the accompanying intact DNA.

3.2. Binding to intact DNA, but not to strand breaks, promotes PARP-1 F1 + F2 sumoylation

In order to explore this phenomenon further, we made use of a structure representing a 5′-phosphorylated SSB flanked by 9 bp of double-stranded DNA and a hairpin on either side of the lesion, thus avoiding the issue of double-stranded termini, to which PARP-1 avidly binds (Fig. 2A). This nicked "dumbbell" accommodates the footprint of PARP-1 on a SSB and is bound by F1 + F2 in a 1:1 stoichiometry with high affinity [18]. When titrated into an F1 + F2 sumoylation reaction in the presence of excess plasmid DNA, the dumbbell at a 1:1 dumbbell:protein ratio significantly inhibited the stimulatory effect of the plasmid (Fig. 2B). Given that the F1 + F2 concentration in this experiment (150 nM) was only slightly above the previously determined K_D for this interaction [18], a moderate excess of ligand would be expected to result in saturation, and indeed we observed full inhibition of sumoylation at a ratio of 2:1 (Fig. 2B). Since the second zinc finger motif (F2) exhibits the highest affinity for SSBs, we examined the effect of DNA on this isolated domain (aa 103–214). As expected, both the enhancement of sumoylation by plasmid DNA and its inhibition by addition of the nicked dumbbell were comparable to the construct containing both zinc finger motifs (Fig. 2C). Introduction of a point mutation, R122I, that abolishes F2 DNA binding without affecting the structural integrity of the domain [18] rendered the sumoylation reaction insensitive to DNA (Fig. 2D). From these observations we conclude that the F2 domain must recognise not only nicks, but also intact DNA, and that of these only the latter promotes sumoylation at K203. Competitive binding of the two forms of DNA, with a strong preference for the nicked form, would then explain why

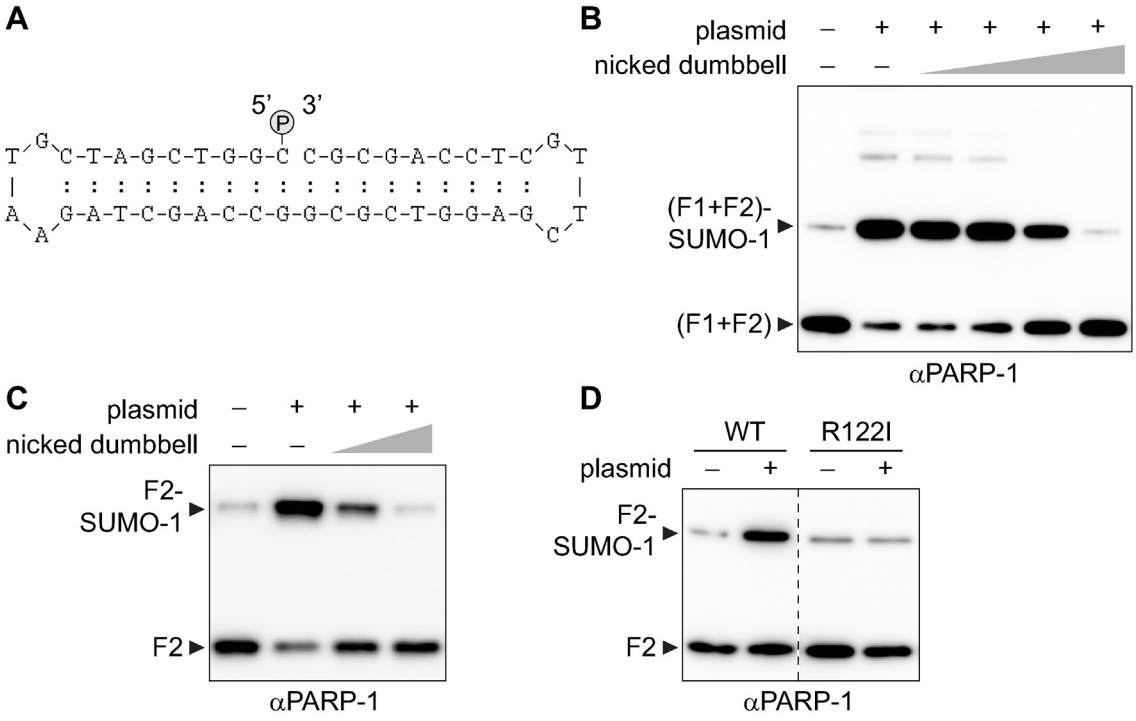

Fig. 2. Sumoylation of F1 + F2 is inhibited by SSBs. (A) Structure of the "nicked dumbbell". (B) Addition of the nicked dumbbell (0.6, 1.5, 3 or 6 pmol) inhibits the stimulatory effect of supercoiled plasmid DNA (240 ng ≈ 360 pmol bp) on the sumoylation of F1 + F2 (3 pmol). (C) The F2 domain (aa 103–214) is sufficient for the stimulatory and inhibitory effects of DNA on sumoylation at K203. *In vitro* sumoylation was performed as in panel B, using 3 pmol F2, 240 ng plasmid DNA and 3 or 6 pmol nicked dumbbell. (D) Inactivation of F2 abolishes the effect of DNA on sumoylation. *In vitro* sumoylation of WT and R122I mutant F2 domain was performed as above.

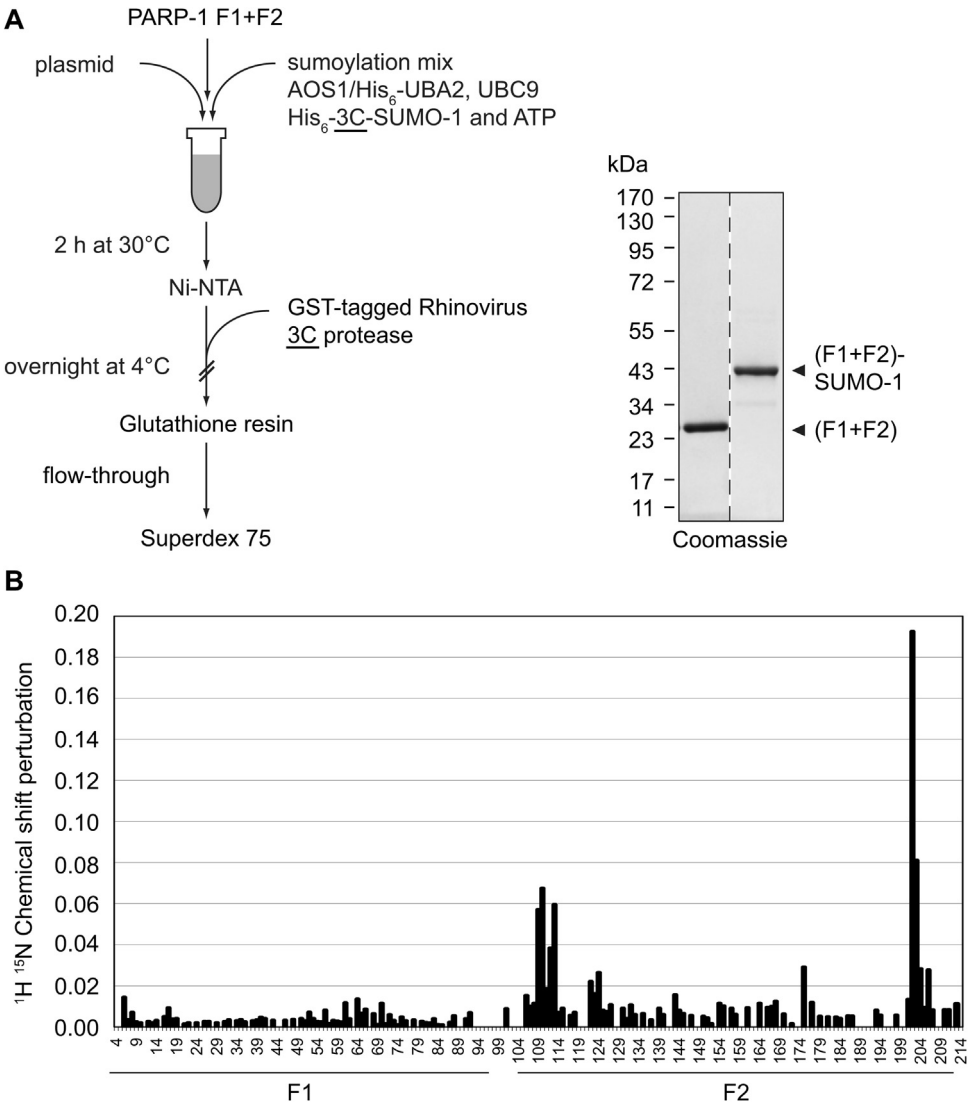

Fig. 3. Sumoylation does not change the properties of PARP-1 F1 + F2. (A) Outline of the purification procedure for sumoylated PARP-1 F1 + F2 and image of a Coomassie-stained gel showing the unmodified protein and the purified product. (B) Histogram of ^{1}H ^{15}N chemical shift perturbations in F1 + F2 upon modification by SUMO-1 at K203. Locations of the F1 and F2 domains are indicated below the residue numbers.

stoichiometric amounts of SSBs inhibit sumoylation even in the presence of a large excess of binding sites on intact DNA.

3.3. Sumoylation does not affect the structure or DNA binding of PARP-1 F1 + F2

Although sumoylation does not affect the DNA-dependent activation of poly-ADP ribose synthesis ([32,34] and our own observations), the proximity of K203 to PARP-1's DNA-binding domain and the impact of DNA on the sumoylation of this residue suggested that the modification might in turn influence the structure and/or DNA-binding properties of F1 + F2. We therefore purified fully sumoylated F1 + F2 to homogeneity (Fig. 3A). Consistent with a previous report [32], the presence of SUMO on K203 did not significantly affect the affinity of F1 + F2 for the nicked dumbbell by electrophoretic mobility shift assays (data not shown). In order to directly examine the effect of SUMO on the structure of free F1 + F2, we modified an ^{15}N- and ^{13}C-labelled version of the protein with unlabelled SUMO-1 and compared the chemical shifts of its backbone amide groups to those of the unmodified domain, using heteronuclear single quantum coherence (HSQC) NMR. As expected, the highest chemical shift perturbations were observed at and immediately around the SUMO attachment site. In addition, some moderate perturbations were found at residues T109-F113, which form a small loop at the extreme N-terminus of the ordered region of F2 (Fig. 3B). It seems likely that these perturbations result from minor changes in hydrogen bonding and ring current effects (from F113), in turn probably reflecting a small change in the conformation of this loop caused by steric interaction with the SUMO moiety. Thus, these data suggest that although SUMO might have a slight preference in the way it positions itself with respect to the F2 domain in the conjugated form, it does not significantly alter the structure of F1 + F2 or make strong non-covalent contacts with any of its residues.

3.4. DNA affects the sumoylation of residues outside F1 + F2

In order to examine whether the striking effect of DNA on the efficiency of PARP-1 sumoylation was relevant in the context of the full-length protein, we performed *in vitro* sumoylation reactions with full-length PARP-1 in the absence and presence of a supercoiled or relaxed plasmid. Addition of DNA resulted in the formation of several high-molecular weight SUMO-1 conjugates that suggested modification of more than one residue (Figs. 4A and

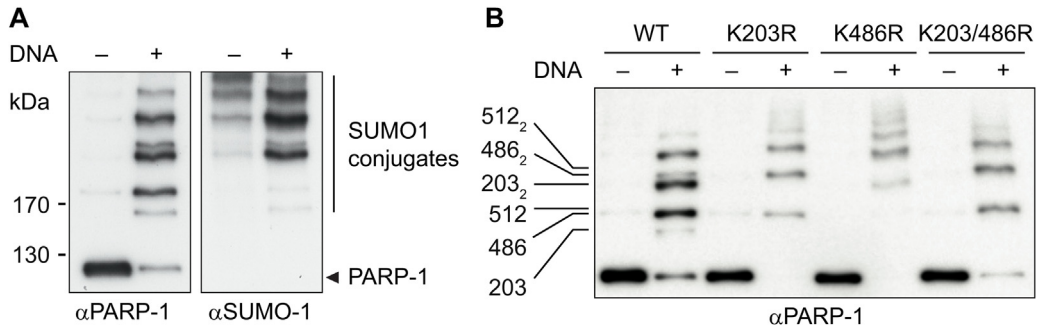

Fig. 4. Full-length PARP-1 is sumoylated at multiple sites. (A) *In vitro* sumoylation of PARP-1 (3 pmol) is stimulated by the addition of supercoiled plasmid DNA (240 ng ≈ 360 pmol bp). Products were detected by anti-PARP-1 and anti-SUMO-1 western blot. (B) *In vitro* sumoylation of WT PARP-1 and the indicated mutants, performed as above.

S4A). Mutation of each of the physiological acceptor sites, K203 and K486, to arginine abolished distinct sets of conjugates, but a series of bands remained even in the K203/486R double mutant (Fig. 4B). Mass spectrometry analysis identified K486 and K512 as sites of SUMO attachment in WT PARP-1 (Fig. S4B and C). K512 conforms to a sumoylation consensus motif (ΨKXE), which may explain why it is targeted by Ubc9. These results demonstrate that the ability of DNA to promote PARP-1 sumoylation is not limited to the SUMO acceptor site immediately adjacent to PARP-1's DBD, but extends to other sites within the protein, including the physiologically relevant K486. Hence, changes in the N-terminal F2 domain upon binding to intact DNA must be transmitted to the rest of the protein.

3.5. Residues outside F1 + F2 contribute to the effect of DNA on PARP-1 sumoylation

As expected from the results obtained with F1 + F2, the nicked dumbbell structure did not enhance the sumoylation of full-length PARP-1, whereas an intact dsDNA dumbbell (Fig. S5) afforded a robust stimulation of the reaction that was dependent on an intact F2 domain (Fig. 5A) and could be inhibited by competition with the nicked form (Fig. 5B).

In order to characterise the requirements for stimulation of PARP-1 sumoylation further, we examined the activities of blunt-ended dsDNA oligonucleotides of different lengths in our *in vitro* assays (Fig. 5C). Stimulation of PARP-1 sumoylation was observed with dsDNA of a minimal length of 15 bp. This could reflect a binding either to the blunt ends or to the duplex itself, which corresponds in size to the footprint of the F2 domain on a SSB (i.e. 7 bp on either side of the break) [21] and should therefore be able to accommodate this domain. An 8 bp duplex did not stimulate sumoylation, although it enhanced PARP-1's catalytic activity as efficiently as longer duplexes (Fig. S6), suggesting that a sufficient stretch of intact dsDNA rather than free ends was required for the stimulation. Duplexes longer than 15 bp were only slightly more efficient, whereas single-stranded oligonucleotides of comparable lengths were inactive. Very long ssDNA (61 nt) again provided an enhancement of PARP-1 sumoylation, but this may well be attributable to regions of secondary structure within the sequence. In contrast to dsDNA, a double-stranded RNA of 21 bp did not stimulate the reaction at all (Fig. 5D), indicating that the effect was specific for DNA.

If the effect of DNA on PARP-1 sumoylation were entirely mediated by the F2 domain, an inactivating mutation in this domain in full-length PARP-1 should abolish all sensitivity of the reaction to DNA, as observed before with the isolated F2 construct. We therefore examined a mutant, R122/138A, which harbours a DNA binding-deficient F2 domain. Surprisingly, we found that while short duplexes had no effect on the mutant, a 61 bp dsDNA stimulated the sumoylation of the mutant protein to the same extent as that of wild-type PARP-1 (Fig. 5E and F). Similarly, plasmid DNA at a non-saturating concentration was equally efficient in enhancing the sumoylation of the mutant and the wild-type protein (Fig. 5G). Hence, long stretches of dsDNA appear to overcome the need for F2, suggesting that DNA probably influences the sumoylation of full-length PARP-1 through regions other than the F2 domain alone. Consistent with this notion, addition of the nicked dumbbell structure, which is specifically recognised by the F2 domain [18], inhibited the stimulatory effect of a 15 bp, but not of a 61 bp duplex (Fig. 5H). This difference was not due to the overall increased amount of DNA in the latter case, as inhibition by the nicked dumbbell was not regained even when the molar amount of the 61 bp duplex was reduced fourfold (Fig. S7).

In order to address the question as to what parts of full-length PARP-1 were involved in reacting to long stretches of dsDNA, we introduced mutations into the F1 and F3 domains, as they had been implicated in the DNA-dependent activation of PARP-1. Although we were unable to obtain an F1 mutant that did not heavily aggregate *in vitro* (data not shown), the F1 motif is unlikely to mediate the length-dependent phenomenon, because in the context of PARP-1 F1 + F2, the nicked dumbbell was capable of inhibiting the stimulation of sumoylation induced by long plasmid DNA (Fig. 2C). Inactivation of F3 by a C298A mutation yielded soluble protein, but to our surprise the mutant behaved very similar to wild-type PARP-1 with respect to the length dependence of stimulation and inhibition of the sumoylation reaction (Fig. S8). If anything, sumoylation of the F3 mutant was slightly more efficient than of the wild-type protein. These results indicate that the F3 motif is not involved in mediating the length-dependent effect of DNA on full-length PARP-1 sumoylation. Hence, it is likely that other regions of the protein, possibly the WGR domain [20], contribute to the recognition of long intact DNA.

3.6. DNA binding enhances PARP-1's affinity for Ubc9

Our observations raised the question as to how DNA enhances PARP-1 sumoylation. Direct binding of the E2, Ubc9, to DNA could be responsible for the stimulatory effect. Alternatively, binding to DNA could trigger a conformational change within PARP-1 leading to an enhanced affinity for the Ubc9-SUMO thioester, either through the exposure of a SUMO interaction motif (SIM) or by means of direct interactions with Ubc9. In order to distinguish between these scenarios, we performed a series of pulldown assays under conditions similar to those that had been found to efficiently stimulate PARP-1 sumoylation. When coupled to streptavidin agarose via a biotin linkage, a 15 bp duplex DNA efficiently retained F1 + F2, but not ^{GST}Ubc9 (Fig. 6A and B), thus ruling out

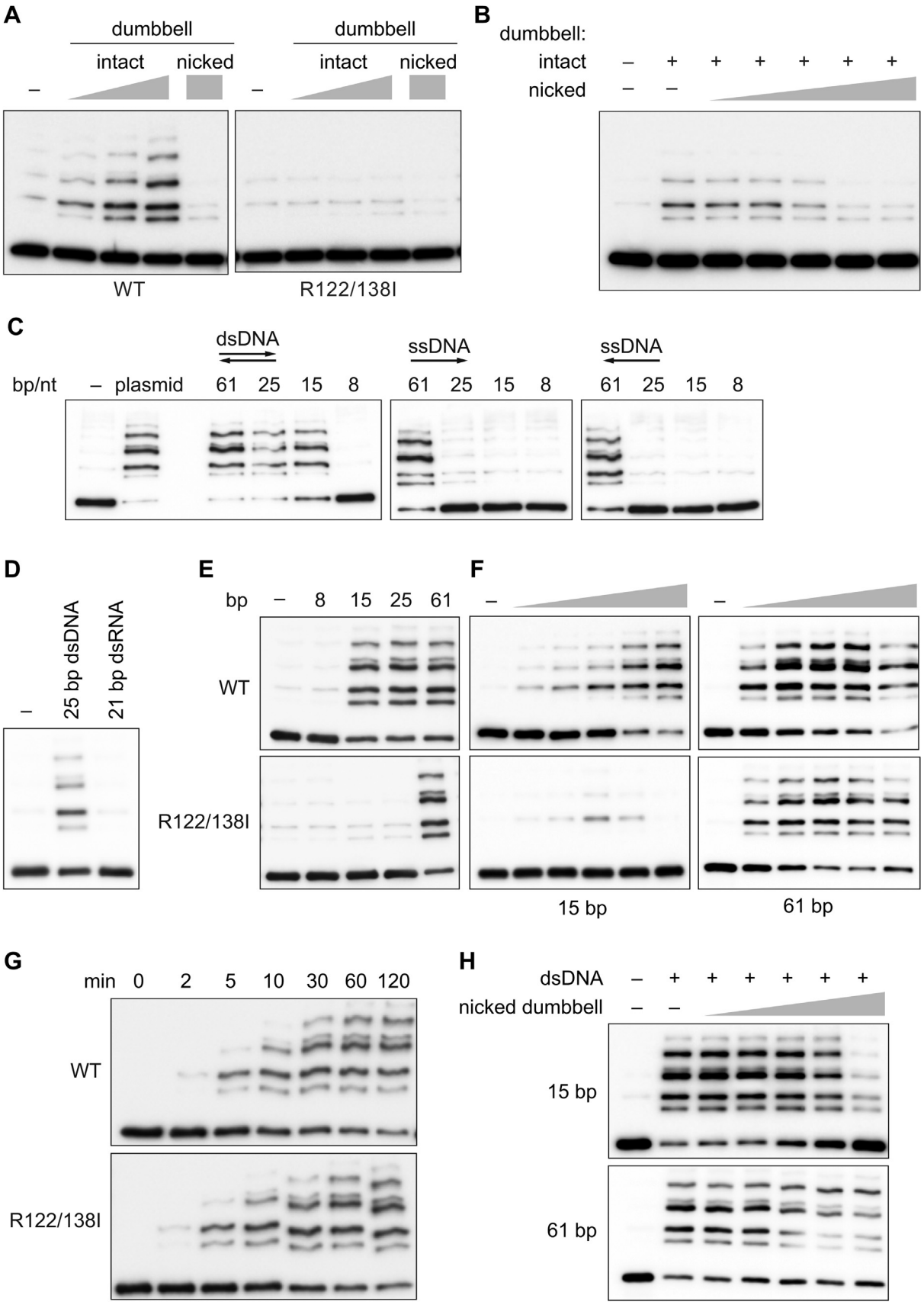

Fig. 5. DNA length affects the efficiency of PARP-1 sumoylation. (A) The F2 domain is required for the effects of intact and SSB-containing DNA on PARP-1 sumoylation. WT and R122/138I mutant PARP-1 (3 pmol) were sumoylated in the presence of nicked (6 pmol) or intact (1.5, 3 or 6 pmol) dumbbell. (B) The nicked dumbbell abolishes the stimulatory effect of the intact dumbbell on PARP-1 sumoylation by competition. The assay was carried out as in panel A with 3 pmol intact dumbbell and increasing amounts of nicked dumbbell (0.38, 0.75, 1.5, 3 and 6 pmol). (C) A minimal length of dsDNA is required for stimulation of PARP-1 sumoylation. Reactions were set up as in panel A, but in the presence of 3 pmol of the indicated oligonucleotides. Plasmid DNA (240 ng) served as a control. (D) dsRNA does not stimulate PARP-1 sumoylation. Reactions contained

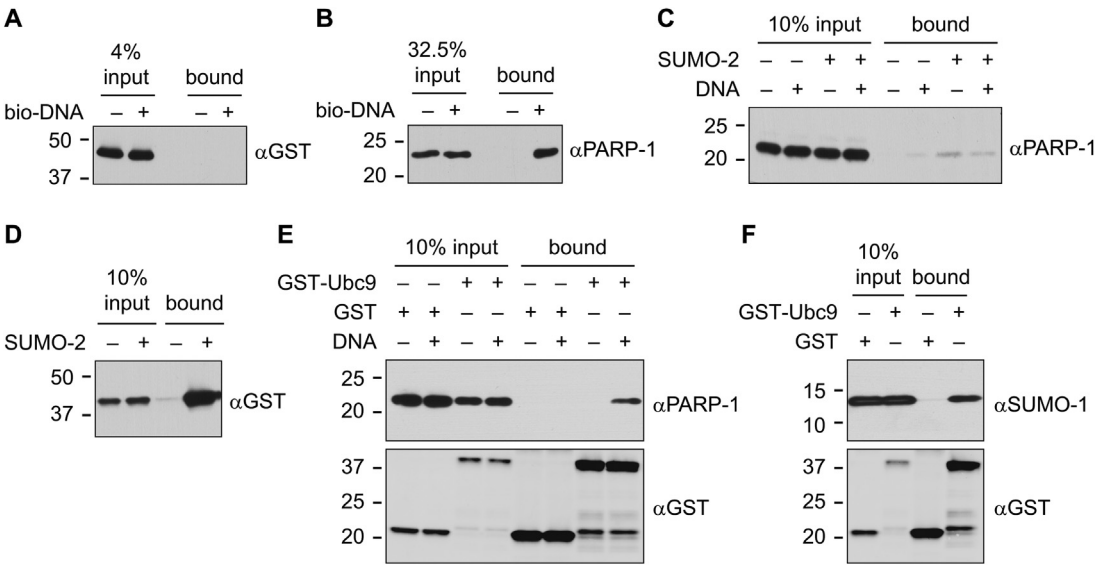

Fig. 6. DNA binding by F1 + F2 promotes interaction with Ubc9. (A) ^{GST}Ubc9 does not bind to a 15 bp duplex DNA immobilised on streptavidin agarose via a biotin linkage. Unlabelled DNA is used as a negative control. (B) Under the same conditions, PARP-1 F1 + F2 binds to the 15 bp duplex. (C) PARP-1 F1 + F2 is not significantly retained by SUMO-2 agarose in the presence or absence of DNA. Underivatised agarose is used as a negative control. (D) ^{GST}Ubc9 binds to SUMO-2 agarose in the absence of DNA. (E) PARP-1 F1 + F2 binds to ^{GST}Ubc9 immobilised on glutathione Sepharose in the presence, but not in the absence of DNA. GST is used as a negative control. (F) SUMO-1 binds to immobilised ^{GST}Ubc9 in the absence of DNA.

a direct interaction between Ubc9 and DNA. Non-covalent interactions between SUMO and F1 + F2 in the presence or absence of plasmid DNA were assayed using SUMO-2 coupled to agarose beads, but no significant retention of the PARP-1 fragment was observed (Fig. 6C), even though Ubc9 efficiently bound to the beads under the same conditions (Fig. 6D). Hence, DNA binding does not lead to the exposure of an obvious SIM. Finally, we used immobilised ^{GST}Ubc9 to examine a potential interaction with F1 + F2. While no binding was detected in the absence of DNA, addition of DNA promoted the retention of F1 + F2 on the beads (Fig. 6E). In contrast, SUMO bound to ^{GST}Ubc9 even in the absence of DNA (Fig. 6F). Given that Ubc9 itself does not bind to DNA, these results suggest that DNA indeed causes a conformational change in F1 + F2 that leads to an enhanced affinity for the E2.

3.7. PARP-1 sumoylation is enhanced by chromatin, but not by DNA damage

In order to assess whether the enhancement of PARP-1 sumoylation by DNA was relevant in the context of chromatin, we examined the ability of a synthetic array of 60 nucleosomes, assembled on an 11 kbp dsDNA, to stimulate the reaction. Fig. 7A shows that the nucleosome array was almost as effective in promoting PARP-1 sumoylation as the corresponding naked dsDNA.

Sumoylation of PARP-1 *in vivo* was detected by co-transfection of mammalian cells with $FLAG_3$-PARP-1 and His_6-SUMO-1. The two major sumoylation sites, K203 and K486, were confirmed by the appearance of two PARP-1-specific conjugates of ca. 150 kDa, which were either abolished or reduced in intensity upon mutation of the respective lysines to arginine (Fig. 7B). However, the modification pattern observed in the K203/486R double mutant indicated that at least one additional lysine was used in the absence of the two major acceptor sites. Although K512 was identified as a relevant site *in vitro* (Fig. S4), the remaining signal in the K486/512R double mutant suggested additional redundancy among the sumoylation sites *in vivo*. K233 and K249 were identified as potential sumoylation sites by bioinformatic analysis, but the modification pattern remained unchanged in the respective mutants.

Sumoylation of PARP-1 was previously reported to be induced by heat shock [33], but the influence of other environmental stimuli, particularly the effect of DNA damage, had not been examined. We therefore determined the extent of endogenous PARP-1 sumoylation in response to a series of stress conditions, including heat, osmotic, ethanol and oxidative stress, by means of Ni-NTA pull-down of His_6-SUMO-3 conjugates from total HeLa cell extracts. While in our hands no significant reaction to heat shock was observed, the extent of PARP-1 sumoylation followed the overall pattern of total SUMO-3 conjugates in that it was enhanced mainly by oxidative and to some extent by osmotic shock (Fig. 7C). As the strong response to oxidative stress could equally be due to protein or DNA damage, we applied a series of other DNA-damaging agents known to cause SSBs and/or DSBs. Importantly, none of them induced PARP-1 sumoylation above the basal level observed in untreated cells (Fig. 7D).

In order to determine whether *in vivo* sumoylation of PARP-1 is at all affected by DNA, we isolated His_6-SUMO-3 conjugates from the soluble and the chromatin-associated fractions of HeLa cell extracts. Sumoylated PARP-1 was exclusively found in the chromatin, whether or not the cells had suffered DNA damage (Fig. 7E). Consistent with our *in vitro* data, this strongly suggested that *in vivo* the reaction also occurs in association with DNA, but is independent of DNA lesions.

3.8. PARP-1 activation and sumoylation are controlled independently in vivo

The question of a possible cross-talk between PARP-1 sumoylation and the enzyme's catalytic activation by DNA damage was

3 pmol PARP-1 and 1 pmol of the indicated oligonucleotides. (E) Sumoylation of an F2 mutant of PARP-1 (3 pmol) is stimulated only by long oligonucleotides (3 pmol). (F) Increasing amounts of the indicated dsDNA oligonucleotide (0.38, 0.75, 1.5, 3 and 6 pmol) were added to *in vitro* sumoylation reactions containing WT or R122/138I mutant PARP-1 (3 pmol). (G) Time course of PARP-1 sumoylation stimulated by 20 ng (≈30 pmol bp) of supercoiled plasmid DNA. (H) The nicked dumbbell (0.38, 0.75, 1.5, 3 and 6 pmol) inhibits the stimulatory effect of short, but not long dsDNA (3 pmol) on the sumoylation of PARP-1 (3 pmol). All blots were developed with an anti-PARP-1 antibody.

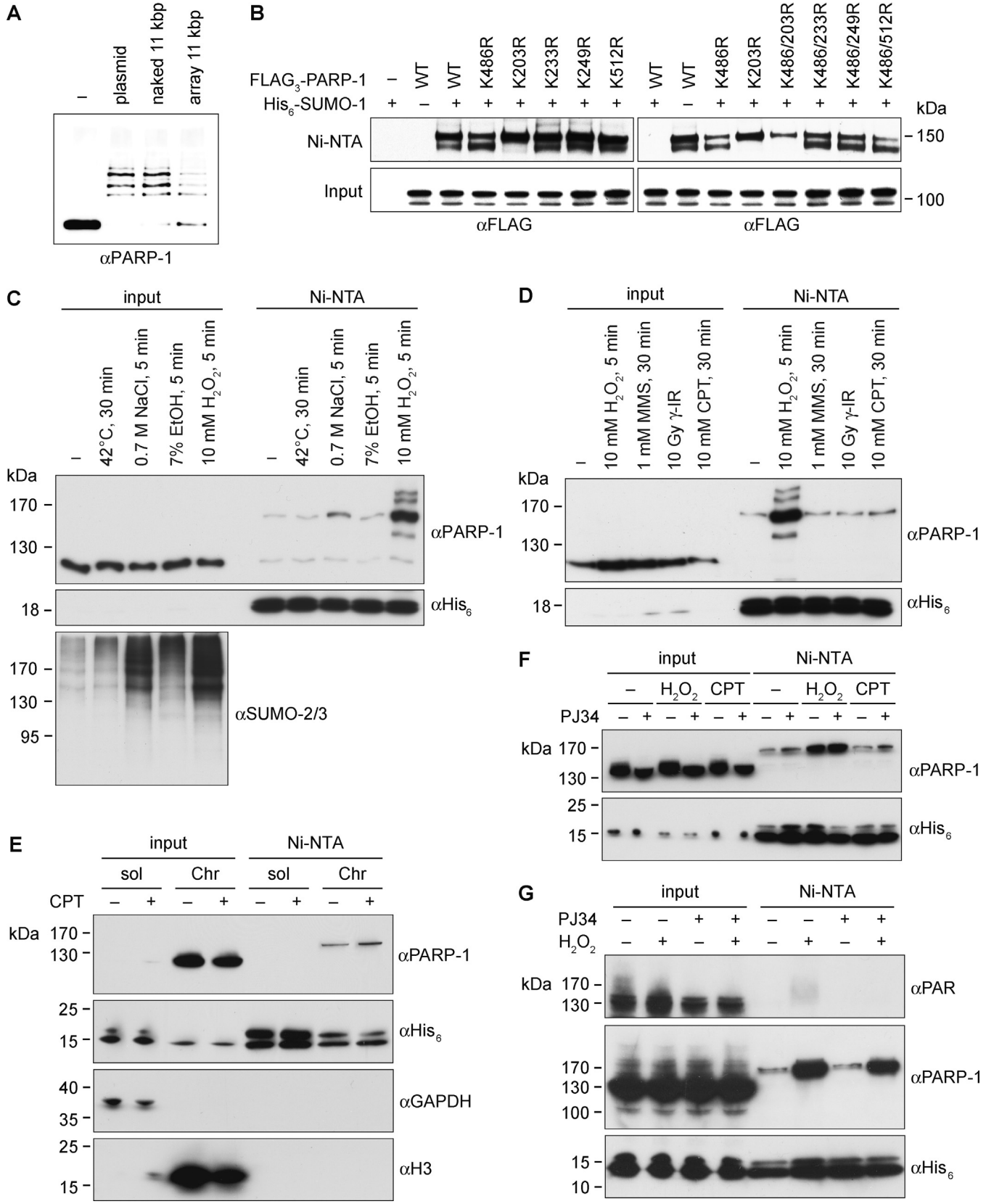

Fig. 7. PARP-1 sumoylation is stimulated by chromatin, but not by DNA damage. (A) *In vitro* sumoylation of PARP-1 (3 pmol) in the presence of 240 ng of plasmid DNA or an 11 kbp duplex, supplied either as unoccupied dsDNA or as an array of 60 nucleosomes. (B) PARP-1 is sumoylated at multiple sites in HEK293 cells transfected with $FLAG_3$-PARP-1 and His_6-SUMO-1. Sumoylated species were isolated by Ni-NTA affinity chromatography, and PARP-1-specific conjugates were detected by anti-FLAG western blot. (C) Sumoylation of endogenous PARP-1 in HeLa cells is stimulated by oxidative stress. HeLa cells producing His_6-SUMO-3 were subjected to the indicated stress conditions, and SUMO-3 conjugates were isolated by Ni-NTA affinity chromatography. (D) Sumoylation of PARP-1 in HeLa cells is not induced by DNA damage. SUMO-3 conjugates were

addressed by means of a PARP-1 inhibitor, PJ34, whose action results in the trapping of the enzyme at strand breaks and preventing its release. Treatment with the inhibitor prevented auto-poly(ADP-ribosylation) of PARP-1, but did not abolish its sumoylation under conditions of either oxidative stress or DNA damage (Fig. 7F). In fact, sumoylation appeared to be slightly enhanced, consistent with the prolonged residence of PARP-1 on DNA upon inhibition.

The notion that PARP-1's catalytic activation appears to be controlled separately from its sumoylation raised the possibility that there might be two distinct populations of PARP-1, involved in damage-induced poly(ADP-ribosylation) and possibly SUMO-mediated transcriptional regulation, respectively. In order to find evidence for or against this scenario, we asked whether the two modifications ever coincide on PARP-1 *in vivo*. Isolation of His_6-SUMO-3 conjugates from H_2O_2-treated cells revealed a faint band of poly(ADP-ribose)-reactive material at a size consistent with doubly modified PARP-1, which disappeared upon treatment with PARP-1 inhibitor (Fig. 7G). Thus, poly(ADP-ribosylation) and sumoylation of PARP-1 are not mutually exclusive, but the degree of overlap appears to be minor. Taken together, these findings suggest that the damage-induced catalytic activation of PARP1 and its sumoylation are mediated by independent regulatory circuits.

4. Discussion

4.1. PARP-1 sumoylation is guided by DNA structure

In this study, we have examined the impact of DNA on the sumoylation of PARP-1 and have delineated which parts of the protein contribute to sensing the nature of the DNA. Our findings suggest that PARP-1 sumoylation is stimulated by regular B-form DNA without any unusual features. The binding of PARP-1 to such DNA has been controversial [16,42–44], but our observation that F2 alone was capable of differentiating between nicked and intact DNA argues in favour of a model where this domain can interact with undamaged DNA. In this mode, the protein was efficiently sumoylated. In contrast, binding to a DNA carrying a SSB did not enhance sumoylation, and due to the much higher affinity of F2 for damaged versus undamaged DNA, small amounts of nicks effectively inhibited sumoylation at K203 even in the presence of a large excess of intact DNA. Binding to intact DNA by F2 is consistent with the structure of this domain, revealing robust contacts between the protein and the uninterrupted sugar-phosphate backbone of a DNA ligand [17]. In the context of the full-length protein, we observed that the critical influence of F2 was overcome by long stretches of dsDNA. Thus, additional domains must also contribute to the recognition of intact DNA. Having excluded the F1 and F3 domains, we consider the WGR domain the most likely candidate, as this was recently shown to make extensive contacts to the sugar-phosphate backbone in the recognition of a DSB [20].

The impact of DNA structure on PARP-1 sumoylation suggests a mechanism of signalling across the protein, where recognition of a small duplex by F2 promotes a conformational change in PARP-1 that causes an enhanced affinity for Ubc9, which may be sufficient to cause sumoylation at lysines situated outside PARP-1's DNA-binding domain, such as K486. This process is reminiscent of the activation of PARP-1's C-terminal catalytic domain by binding of its N-terminal region to damaged DNA. The crystal structure of selected PARP-1 domains in complex with a DSB [20] has given insight into how this activation is accomplished: cooperation between the F1, F3 and WGR domains in DNA binding causes them to collapse onto the DNA, leading to a significant perturbance of the catalytic domain and thereby its activation. A similar mechanism could in principle apply to the stimulation of PARP-1 sumoylation. However, F2 is dispensable for catalytic activation of the enzyme in response to DSBs, while sumoylation was found to be controlled mainly by F2, but was unaffected by F1 and F3. Hence, the way in which DNA structure influences PARP-1 conformation and accessibility likely differs between the two phenomena. Structures of PARP-1 bound to SSBs and most importantly to intact DNA may provide further insight.

4.2. Independent regulation of PARP-1 sumoylation and catalytic activation

PARP-1 contributes to a variety of DNA-associated processes, ranging from DNA repair to a modulation of chromatin structure [12,14,15]. Strand breaks activate PARP-1's enzymatic activity, which leads to the modification of chromatin-associated proteins and PARP-1 itself [12]. Conversely, we now show that binding to intact DNA strongly stimulates PARP-1 sumoylation, which has in turn been implicated in transcriptional regulation [32,33]. Consistent with these *in vitro* data, sumoylated PARP-1 is found in association with chromatin, but the modification is not enhanced by DNA damage. Based on these observations, we propose that the way in which PARP-1 recognises DNA may influence whether it is modified by SUMO or not, thus providing a means to delineate two distinct functionalities of the enzyme.

In the context of the DNA damage response, synthesis of poly(ADP-ribose) is believed to cause a general decompaction of chromatin to provide access to the site of damage, and it facilitates the recruitment of specific repair factors [26–30]. In this situation, PARP-1's catalytic activity is stimulated through its binding to damaged DNA, but sumoylation would not be required. Although we observed that DNA strand breaks do not directly inhibit sumoylation of the full-length protein when a sufficient stretch of dsDNA is available, PARP-1's rapid auto-poly(ADP-ribosylation) upon binding to damaged DNA would likely prevent extensive sumoylation *in vivo* by causing the release of the enzyme from DNA, thus removing the stimulus [15].

Transcriptional regulation often appears to involve a modulation of chromatin structure via poly(ADP-ribose) synthesis as well [25]. Yet, the enzyme can also act more directly as a co-regulator, which would involve an association with intact DNA, but not necessarily its catalytic activity [31]. Particularly this latter aspect of PARP-1 function might be subject to modulation by SUMO, as suggested by the finding that the modifier interferes with the enzyme's function as a co-activator of HIF1-α [32]. In this or a similar context, stimulation of PARP-1 sumoylation by association with intact DNA would help to direct the modification to chromatin-associated molecules. Hence, the influence of DNA structure on sumoylation efficiency could serve as an elegant mechanism to limit PARP-1 sumoylation to the appropriate locations. As we have shown in Fig. 6G, automodification and sumoylation are not mutually exclusive; hence, there may be situations in which both modifications work hand in hand. For example, a recent study reported a rapid, heat shock-induced activation of PARP-1 at the HSP70 promoter [45], where PARP-1 sumoylation was also observed to impinge

isolated and detected as above after treating the cells as indicated. (E) Sumoylated PARP-1 is associated with chromatin *in vivo*. Total HeLa cell lysates were fractionated in soluble and chromatin-associated fractions, and PARP-1 SUMO conjugates were detected in the fractions by Ni-NTA pulldown. GAPDH and histone H3 served as controls for soluble and chromatin-associated proteins, respectively. (F) PARP-1 inhibition does not influence PARP-1 sumoylation. SUMO-3 conjugates were isolated after the indicated treatments with PARP-1 inhibitor PJ34 as described for panel C. (G) PARP-1 can be simultaneously auto-poly(ADP-ribosylated) and sumoylated. SUMO-3 conjugates were isolated after the indicated treatments as above, and doubly modified species were detected by anti-poly(ADP-ribose) (PAR) western blot.

on transcription [33]. However, a detailed analysis of the kinetics of PARP-1 modifications at specific promoters will be required to determine the relationship between automodification and sumoylation in transcriptional control.

4.3. Structure of the PARP-1 SUMO conjugate and its functional implications

The absence of major chemical shift differences outside the region surrounding the SUMO acceptor site, K203, between sumoylated and unmodified F1 + F2 indicates that the modification does not induce major changes in the conformation of PARP-1's DNA-binding domain. In this respect, the structure of sumoylated F1 + F2 resembles those of other covalent SUMO conjugates, which mostly adhere to a "beads-on-a-string" model where SUMO is flexibly tethered to its substrate without extensive non-covalent contacts or changes to the conformations of the target protein or SUMO itself [46–50]. Consistently, sumoylation changes neither the affinity of F1 + F2 for DNA nor the catalytic activity of the full-length protein. These observations suggest a uni-directional influence of DNA on PARP-1 sumoylation. Rather than directly modifying PARP-1's properties, SUMO is therefore likely to function by creating an additional or blocking an existing interaction surface for downstream effector proteins. The two effects are not mutually exclusive, and there is evidence that both might apply in the case of PARP-1: in the context of hypoxia-responsive genes, SUMO was shown to prevent PARP-1 acetylation through interference with p300 binding, thereby reducing transcriptional activity [32]. In response to heat shock, SUMO was instead proposed to promote the recognition of PARP-1 by the SUMO-targeted ubiquitin ligase RNF4 and contribute to the activation of *HSP70.1* transcription by facilitating proteasomal turnover of PARP-1 [33]. Our own data support this scenario: we found that mutating the main sumoylation sites of PARP-1 *in vivo* stabilised the enzyme to a similar extent as treatment with the proteasome inhibitor MG132 (Fig. S8). Whether there is a common principle as to how PARP-1 sumoylation impinges on transcription has yet to be established. A systematic analysis of the effect of SUMO on PARP-1-responsive genes is expected to provide the necessary mechanistic insight.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dnarep.2013.07.001.

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